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RESEARCH ARTICLE

A REVIEW ON APPROACHES TO DEVELOP PLANT GROWTH PROMOTING RHIZOBACTERIA

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ABSTRACT

Plant growth promoting rhizobacteria (PGPR) has been in limelight among agriculturists for their benefits on crop. Several scientists have followed multidisciplinary approaches to enhance the effectiveness of PGPR. Variety of mechanisms are involved which help in increasing the plant growth and productivity. These mechanisms include nitrogen fixation, phosphate solubilization, siderophore production, production of plant growth hormones, Volatile organic compound production, exhibiting antifungal activity, beneficial synergistic effects etc. Lack of understanding of mechanisms involved in the effects of PGPR has often given inconsistent results in the terms of crop yield. In this review we will discuss the examples of organisms used as PGPR, their mechanisms and modern approaches that can be applied before commercializing the PGPR.

INTRODUCTION

PGPR have gained importance in agriculture as these are environment friendly and are desired for sustainable agricultural practices. A great variety of organisms have been used as PGPR, Rhizobia are the most common. Rhizobia along with other co-inoculants are widely used because of comparatively high yield increases. Co-inoculants tested with rhizobia include strains of *Azospirillum*, *Azotobacter*, *Bacillus*, *Pseudomonas*, *Serratia* and *Streptomyces* (Baudoin *et al.*, 2010; Biari *et al.*, 2008; Ahmad *et al.*, 2008). Field trials conducted in India showed that depending on the legume, soil and agroclimatic conditions nearly 50% of nitrogenous fertilizer could be saved through rhizobial inoculations with considerable increase in yield (Rewari, 1988 and Tilak, 1993). *Rhizobium*, when co-inoculated with Phosphate Solubilising Bacteria (PSB) has revealed synergistic effect on symbiotic parameters and grain yield. Phosphate solubilizing bacteria improves the competitive ability and symbiotic effectiveness of inoculated *Rhizobium* sp. in lentil under field conditions (Kumar *et al.*, 2008). The single and dual inoculation *Rhizobium* and phosphorus (P) solubilising bacteria with fertilizer (P_2O_5) significantly increases root and shoot weight, plant height, spike length, grain yield, seed P content, leaf protein and leaf sugar content of the wheat crop in a P deficient natural non-sterilized sandy loam soil and is 30-40% better than only P fertilizer for improving grain yield (Afzal *et al.*, 2008). The P-solubilising strains and the N2-fixing bacterial strains have great potential in being formulated and used as biofertilizers (Cakmakc, 2007a).

The species of *Pseudomonas* are predominant in the rhizosphere regions of different crops. *Pseudomonas* strains are effective PGPR as they exhibit a wide range of properties *viz.* production of phytohormones like indoleacetic acid (IAA), gibberellic acid and cytokinins; phosphate solubilization and other nutrients (Vyas *et al.*, 2009); Siderophore production, antibiotics such as 2,4-diacetylphloroglucinol (2,4-DAPG), phenazines, pyrrolnitrin and pyoluteorin, surface-active antibiotics, biocides such as hydrogen cyanide (HCN) (Raaijmakers *et al*., 2002) and cell wall lytic enzymes (Haas *et al.*, 2005). In addition, pseudomonads are reported to produce the enzyme, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, which plays an important role in maintaining ethylene levels in plant tissues under biotic and abiotic stresses (Penrose *et al*., 2003). Studies have demonstrated that the PGPR can stimulate plant growth through the production of auxins; indole acetic acid (IAA) (Spaepen *et al*., 2008), gibberellins (Bottini *et al*., 2004) and cytokinins (Timmusk *et al*., 1999), or by regulating the high levels of endogenous ethylene in the plant (Glick *et al*., 1998).There are several organisms which by one or the other method are PGPRs. These organisms have been used as inoculants for the specific plant species and improved growth has been reported.

Mechanisms of PGPR

Nitrogen fixation is one of the most common and well known methods that help in growth enhancement. Symbiotic and asymbiotic nitrogen fixers make nitrogen available to the plants. Phosphate solubilizing organisms help in mobilizing inorganic phosphate and as Nitrogen and phosphorus are two essential nutrients required for plant growth, their conjunctive use is always beneficial. There are reports of Pseudomonads being used as co-inoculants and increased yield of crops. FLPs help in the maintenance of soil health and are metabolically and functionally most diverse (Lata *et al*., 2002 and Lugtenberg *et al*., 1999). The presence of *Pseudomonas fluorescence* inoculants in the combination of microbial fertilizer plays an effective role in stimulating yield and growth traits of chickpea (Rokhzadi *et al.*, 2008). Isolates of Fluorescent Pseudomonads (FLPs) from roots, shoots, and rhizosphere soil of sugarcane provides significant increases in fresh and dry masses (Mehnaz *et al.*, 2009). Field trials of a pseudomonad strain (GRP3) lead to a great increase in

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yield of legumes (Johri BN, 2001). A secondary metabolite produced commonly by rhizosphere pseudomonads is Hydrogen Cyanide (HCN), a gas known to negatively affect root metabolism and root growth (Schippers *et al.,* 1990) and is a potential and environmentally compatible mechanism for biological control of weeds (Heydari *et al.,* 2008). The HCN production is found to be a common trait of *Pseudomonas* (88.89%) and *Bacillus* (50%) in the rhizospheric soil and plant root nodules (Ahmad *et al*., 2008 and Charest *et al.,* 2005) and is a serious environmental pollutant and a biocontrol metabolite in *Pseudomonas* species. It was previously not known if glycine was a carbon precursor for HCN in *Pseudomonas aeruginosa* (Saharan and Nehra, 2011).

Some of the recent studies have indicated that the *Pseudomonas* spp. metabolites such as HCN may enhance plant establishment. Wani *et al.,* (2007) tested the rhizosphere isolates for HCN producing ability *in vitro* to find that most of the isolates produced HCN and helped in the plant growth. The isolates from the rhizospheric soil of chickpea also exhibits more than two or three PGPR traits including HCN production, which promotes plant growth directly or indirectly or synergistically (Joseph et al., 2007).The entomopathogenic bacterium *Pseudomonas entomophila* produces HCN which is a secondary metabolite and is implicated in biocontrol properties and pathogenicity exerted by other bacteria (Ryall *et al.,* 2009). The *Pseudomonas fragi* CS11RH1 (MTCC 8984), a psychrotolerant bacterium produces hydrogen cyanide (HCN) and the seed bacterization with the isolate significantly increases the percent germination, rate of germination, plant biomass and nutrient uptake of wheat seedlings (Selvakumar *et al.,* 2009). *Pseudomonas* sp. has the capacity to utilize siderophores produced by diverse species of bacteria and fungi, and *Pseudomonas putida* can utilize the heterologous siderophores produced by rhizosphere microorganisms to enhance the level of iron available to it in the natural habitat (Loper *et al*., 1999). Some PGPR synthesize antifungal antibiotics, e.g. *P. fluorescens* produces 2, 4-diacetyl phloroglucinol which inhibits growth of phytopathogenic fungi (Nowak-Thompson *et al.,* 1994).

Certain PGPR degrade fusaric acid produced by *Fusarium* sp. causative agent of wilt and thus prevents the pathogenesis (Toyoda *et al*., 1991). Some PGPR can also produce enzymes that can lyse fungal cells. For example, *Pseudomonas stutzeri* produces extracellular chitinase and laminarinase which lyses the mycelia of *Fusarium solani* (Mauch *et al*., 1988). In recent years, *fluorescent Pseudomonas* has been suggested as potential biological control agent due to its ability to colonize rhizosphere and protect plants against a wide range of important agronomic fungal diseases such as black root-rot of tobacco (Voisard *et al.,* 1989), root-rot of pea (Papavizas *et al*., 1974), root-rot of wheat (Garagulia *et al.,* 1974), damping-off of sugar beet (Fenton *et al*., 1992, Shanahan *et al*., 1992 and Kumar *et al*., 2002) and as the prospects of genetically manipulating the producer organisms to improve the efficacy of these biocontrol agents (Dowling *et al.,* 1994).The enzyme rhodanese from cyanogenic bacterium *Pseudomonas aeruginosa* involved in transfer reactions causes cyanide detoxification (Cipollone *et al.*,2008). The enzymes like chitinase, β-1, 3 Glucanase and Cellulase are involved in antagonistic action of *Pseudomonas* against fungal pathogens (Saraf *et al.,* 2008).The enzyme formamide hydro-lyase is involved in HCN detoxification in sorghum infected by *Gloeocercospora sorghi* (Myers *et al*., 1978). The HCN synthase which produces HCN is encoded by three biosynthetic genes (*hcnA*, *hcnB*, and *hcnC*), but little is known about the diversity of these genes in *fluorescent Pseudomonas* spp and in other bacteria (Ramette *et al*., 2003). According to Glick *et al.,* (1999) the general mechanisms of plant growth promotion by PGPR includes associative nitrogen fixation, lowering of ethylene levels, production of siderophores and phytohormones, induction of pathogen resistance, solubilization of nutrients, promotion of mycorrhizal functioning, decreasing pollutant toxicity etc.

Castro *et al.,* (2009) suggested that PGPR strains can promote plant growth and development either directly and indirectly.
PGPR may use more than one of these mechanisms to enhance plant growth as experimental evidence suggests that the plant growth stimulation is the net result of multiple mechanisms that may be activated simultaneously (Martinez-Viveros *et al.,* 2010). Recently, biochemical and molecular approaches are providing new insight into the genetic basis of these biosynthetic pathways, their regulation and importance in biological control (Joshi *et al.,* 2011). The event of ISR Induced systemic resistance has been demonstrated in various plants inoculated with different species of rhizobacteria (Liu *et al*., 1995; Raj *et al*., 2003; Halfeld-Vieira *et al*., 2006). The ISR occurs when plants previously exposed to biotic and abiotic agents are induced to defense against pathogens, which are spatially separated from the inducer agent (Pieterse *et al.,* 1999; Stadnik 2000). The major indirect mechanism of plant growth promotion in rhizobacteria is through acting as biocontrol agents (Glick, 2012). In general, competition for nutrients, niche exclusion, induced systemic resistance and antifungal metabolites production are the chief modes of biocontrol activity in PGPR (Lugtenberg *et al.,* 2009). Many rhizobacteria have been reported to produce antifungal metabolites like, HCN, phenazines, pyrrolnitrin, 2,4-diacetylphloroglucinol, pyoluteorin, viscosinamide and tens in (Bhattacharyya *et al.,* 2012). Interaction of some rhizobacteria with the plant roots can result in plant resistance against some pathogenic bacteria, fungi, and viruses the phenomenon called induced systemic resistance (ISR) (Lugtenberg *et al.,* 2009).

Approaches to Develop PGPR

The traditional approaches in developing PGPR are screening of these organisms from the rhizosphere and non- rhizosphere soil and studying its effect on plant in the lab. The modern approaches can help enhance the results as stipulated by the traditional approach. Rhizosphere management will require consideration of soil and crop cultural practices as well as inoculant formulation and delivery (Bowen *et al*., 1999, McSpadden *et al.,* 2002). Genetic manipulation of host crops for root-associated traits to enhance establishment and proliferation of beneficial microorganisms (Mansouri *et al*., 2002, Smith *et al*., 1999) is being pursued. The use of multi-strain inocula of PGPR with known functions is of interest as these formulations may increase consistency in the field (Jetiyanon *et al.,* 2002, Siddiqui *et al.,* 2002). The application of molecular tools is enhancing our ability to understand and manage the rhizosphere and will lead to new products with improved effectiveness (Nelson, 2004). Engineering the rhizosphere of crops to improve productivity and plant health has been studied through a number of mechanisms, including manipulating the plants to: modify their rhizosphere to promote nutrient availability, suppress pathogens, or encourage PGPR bacterial growth (Ryan *et al.,* 2008). Sundheim *et al.,* (1988) observed that an engineered strain of Pseudomonas expressing chitinase gene from *Serratia marcescens* more effectively controlled *Fusarium oxysporum* f. sp. redolens and

Gaeumannomyces graminis var. tritici in vitro. Recently, experiments targeting on the DAPG-producing PGPR strain, Pseudomonas fluorescens have demonstrated that plant species can differentially enrich and support different microbial populations (De La Fuente *et al.,* 2006) and genotypes (Landa *et al.,* 2006) in the rhizosphere. Notz *et al.,* (2001) significantly correlated DAPG accumulation by *Pseudomonas fluorescens* CHA0 with the expression of DAPG biosynthesis gene phlA and observed that the expression was significantly greater in the rhizosphere of monocots than dicots. Although the exact mechanism is not totally understood, Di Gregorio *et al.,* (2006) noticed a combined application of Triton X-100 and Sinorhizobium sp. Pb002 inoculums for the improvement of lead phytoextraction by *Brassica juncea* in EDTA amended soil.

CONCLUSION

Most studies on PGPR focus on individual mechanisms, the knowledge of contributory mechanisms can help enhance the effectiveness of commercial PGPR. In years to come scientists will use omics technology to reveal the mechanisms of signal transductions during the conjunctive growth of organisms and biocontrol mechanisms by which plants protect them by enemy attack. The plant growth promoting phenomenon can be attributed to the ability of the isolate to produce IAA, as IAA positively influences root growth and development, thereby enhancing nutrient uptake (Khalid *et al.,* 2004). It is a well-established fact that improved phosphorous nutrition influences overall plant growth and root development (Jones *et al.,* 1994). Siderophore production by the isolate assumes significance for iron nutrition of plants grown under iron deficient conditions (Pieterse *et al.,* 2001) as well inhibition of phytopathogens. Worldwide, there is a profound need to explore varied agro-ecological niches for the presence of indigenous beneficial micro-organisms. With increasing awareness about the problems of chemical fertilizers based agricultural practices (Ahmed, 1995), it is important to search for region-specific microbial strains which can be used as a potential plant growth promoter to achieve desired production of strains.16srRNA sequence analysis is also being used to identify the strains for use at commercial scale.

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